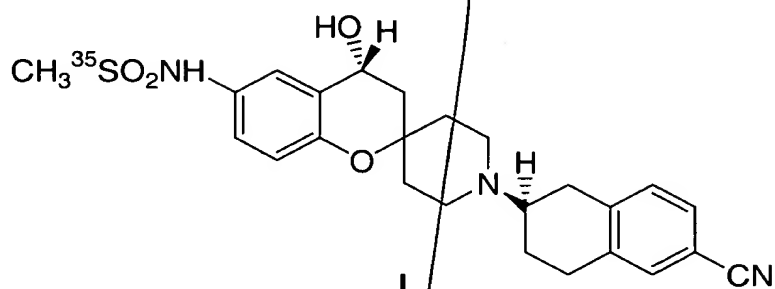


WHAT IS CLAIMED IS:

1. A radioligand compound of Formula I which is



- 5 or pharmaceutically acceptable salts thereof.

2. The radioligand compound of Formula I, as recited in Claim 1, which possesses a specific activity of greater than 500 Ci/mmol.

- 10 3. The radioligand compound of Formula I, as recited in Claim 1, which possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

- 15 4. A method of characterizing an ion channel as an I_{K_R} channel comprising contacting the ion channel with the radioligand compound of Claim 1 and determining if the radioligand compound binds to the ion channel.

- 20 5. A method for characterizing the activity of a compound as an I_{K_R} channel blocker comprising contacting the test compound with a membrane containing the I_{K_R} channel in the presence of the radioligand compound of Claim 1 and monitoring whether the test compound influences the binding of the radioligand compound to the membrane containing the I_{K_R} channel.

- 25 6. The method as recited in Claim 5, wherein the membrane containing the I_{K_R} channel is derived from a cell line transfected with the ERG gene.

7. The method as recited in Claim 6, wherein the cell line is HEK 293 cells or CHO cells.

8. The method as recited in Claim 7, wherein the ERG gene is human, canine or primate.

9. The method as recited in Claim 8, wherein the radioligand compound of Formula I possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

10. A method for assessing the binding of a test compound to a membrane containing the I_{K_r} channel using a radioligand compound of Formula I, $[^{35}\text{S}]$ -radiolabeled (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxy-spiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide, comprising the steps of:

- 1) preparing solutions of the test compound at 5 or more different concentrations, a solution of control vehicle and a solution of (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxy-spiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide (compound of Formula II) in a solvent;
- 2) mixing the radioligand compound of Formula I with the membrane containing the I_{K_r} channel diluted with an assay buffer to form a membrane/radioligand mixture of known concentration;
- 3) incubating a quantity of known concentration of the membrane/radioligand mixture with the solution of test compound, control vehicle or compound of Formula II, as recited in Step 1, for a set time period at a temperature range of between about 40°C and about 37°C to give a mixture of membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II, where the final concentration of the membrane containing the I_{K_r} channel is predetermined;
- 4) isolating from the incubated mixture the membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II;
- 5) measuring the radioactivity of the isolated membrane bound with the radioligand and the test compound, the control vehicle or the compound of Formula II;

- 6) repeating steps 3 through 5 with the test compound at each concentration, the solution of control vehicle and the solution of the compound of Formula II, as recited in Step 1; and
- 7) calculating the IC₅₀ corresponding to the measured radioactivity of: 1) the membrane bound with the radioligand and each concentration of the test compound, 2) the membrane bound with the radioligand and with the control vehicle, and 3) the membrane bound with the radioligand and the compound of Formula II.

11. The method as recited in Claim 10, wherein the membrane containing the I_{Kr} channel is derived from a cell line transfected with the ERG gene.

12. The method as recited in Claim 11, wherein the cell line is HEK 293 cells or CHO cells.

13. The method as recited in Claim 12, wherein the ERG gene is human, canine or primate.

14. The method as recited in Claim 13, wherein the solutions of the test compound are prepared in Step 1 at 7 different concentrations.

15. The method as recited in Claim 14, wherein the time period for incubation in Step 3, is about 30 minutes to 1 hour.

16. The method as recited in Claim 15, wherein the temperature for the incubation in Step 3, is room temperature (25°C).

17. The method as recited in Claim 16, wherein the membrane-bound with radioligand or test compound is isolated in Step 4 with Unifilters, Scintillation Proximity Assay (SPA) beads or the Flashplates.

18. The method as recited in Claim 17, wherein the membrane containing the I_{Kr} channel is derived from a HEK 293 cell line transfected with the human ERG gene.

19. The method as recited in Claim 8, wherein the radioligand compound of Formula I possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

20. A method for assessing the binding of a test compound to a membrane containing the I_{K_r} channel using a radioligand of Formula I, [^{35}S]-radiolabeled (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxy]spiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide, comprising the steps of:

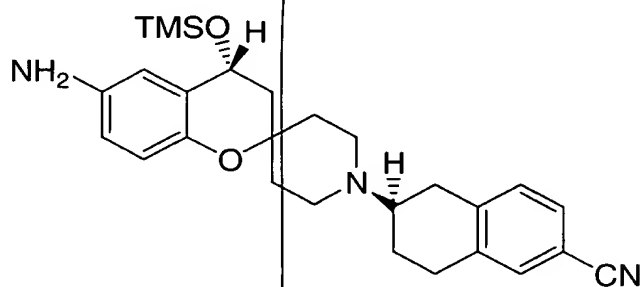
- 1) preparing assay wells with 4 μl of the test compound in dimethylsulfoxide (DMSO) diluted 100x with assay buffer at 5 or more different concentrations, a control vehicle of DMSO and a DMSO solution of (+)-N-[1'-(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxy]spiro[2H-1-benzopyran-2,4'-piperidin]-6-yl]-methanesulfonamide (compound of Formula II);
- 2) adding the radioligand compound of Formula I at 50pM to the membrane containing the I_{K_r} channel diluted with assay buffer to form a membrane/radioligand mixture;
- 3) incubating each assay well with 400 μl of the 50 pM membrane/radioligand mixture for about 75 minutes to about 90 minutes at room temperature (25°C) to give assay wells containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II where the final concentration of the membrane containing the I_{K_r} channel is 11 $\mu\text{g}/\text{ml}$;
- 4) filtering the incubated assay wells through 0.1% BSA presoaked filters to isolate on the filters the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II;
- 5) washing each of the filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II about 5 times with 500 μl of ice cold wash buffer;

- 6) drying the washed-filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II at room temperature in a fume hood;
- 5 7) adding 50 µl Microscint-20 microscintillate to the dried-filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II;
- 10 8) measuring the microscintillation count of the microscintillation-treated filters containing the membrane bound with the radioligand and the test compound at each concentration, the DMSO control vehicle or the compound of Formula II for one minute; and
- 15 9) calculating the IC₅₀ corresponding to the measured microscintillation count of: 1) the microscintillation-treated filters containing the membrane bound with the radioligand and each concentration of the test compound, 2) the microscintillation-treated filters containing the membrane bound with the radioligand and with the control vehicle, and 3) the microscintillation-treated filters containing the membrane bound with the radioligand and the compound of Formula II.
- 20
21. The method as recited in Claim 20, wherein the membrane containing the I_{Kr} channel is derived from a cell line transfected with the ERG gene.
- 25 22. The method as recited in Claim 21, wherein the cell line is HEK 293 cells.
23. The method as recited in Claim 22, wherein the ERG gene is human or canine.
- 30 24. The method as recited in Claim 23, wherein the solutions of the test compound are prepared in Step 1 at 7 different concentrations.
25. The method as recited in Claim 24, wherein the membrane containing the I_{Kr} channel is derived from a HEK 293 cell line transfected with the human ERG gene.
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26. The method as recited in Claim 25, wherein the radioligand compound of Formula I possesses a specific activity of in the range of about 900 Ci/mmol. to about 1498 Ci/mmol.

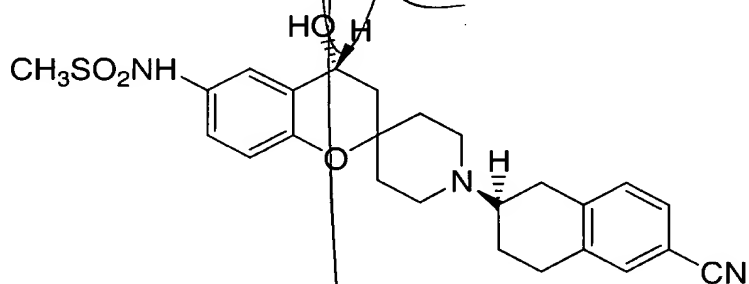
27. The method as recited in Claim 26, wherein the membrane-bound with radioligand or test compound is filtered in Step 4 with Unifilters.

28. A process for the preparation of

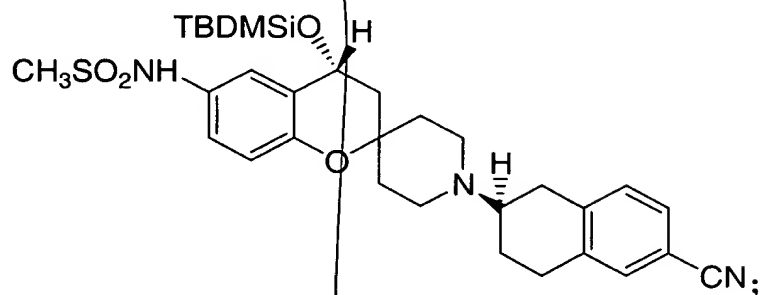


comprising the steps of

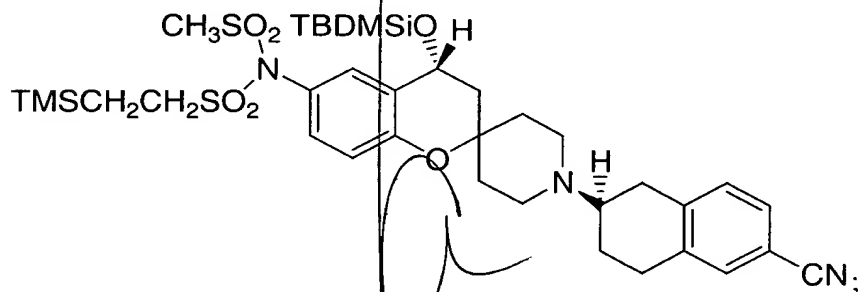
(1) reacting the alcohol with 2,6-lutidine and t-butyldimethylsilyl triflate



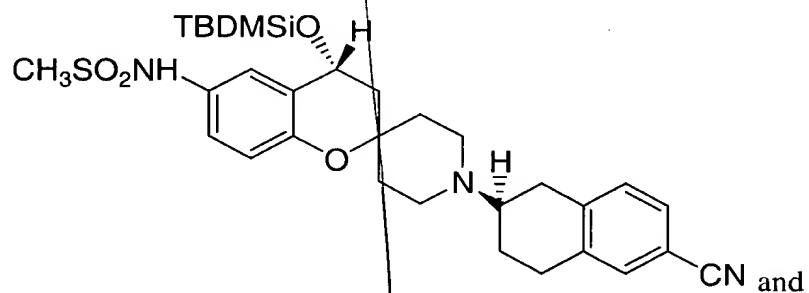
to give a t-butyldimethylsilyl-protected hydroxyl compound

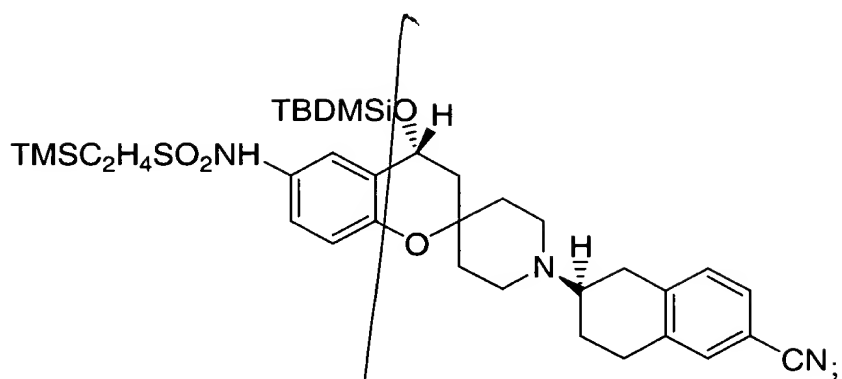


(2) alkylating the t-butyldimethylsilyl-protected hydroxyl compound by treating with sodium hydride, and then treating with 2-trimethylsilylethanesulfonylchloride to give the disubstituted sulfonamide compound

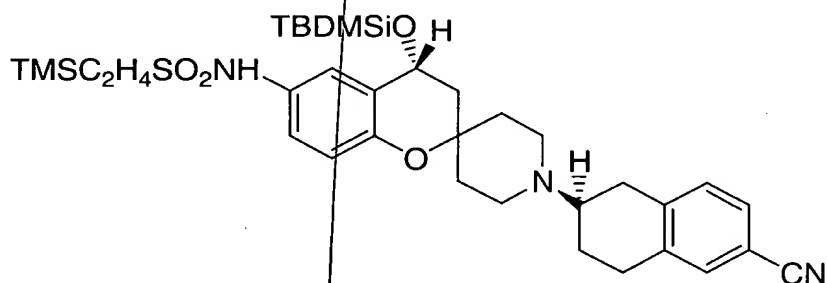


(3) treating the disulfonamide with an C₁-C₈-alkanethiolate to give a mixture of the following sulfonamides;



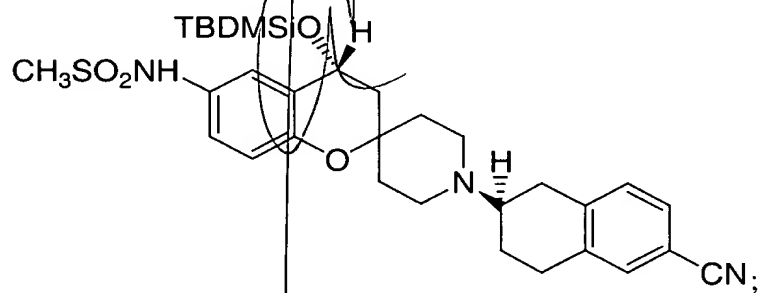


(4) separating the sulfonamide mixture using chromatography to isolate the non-polar sulfonamide eluting with a non-polar solvent system

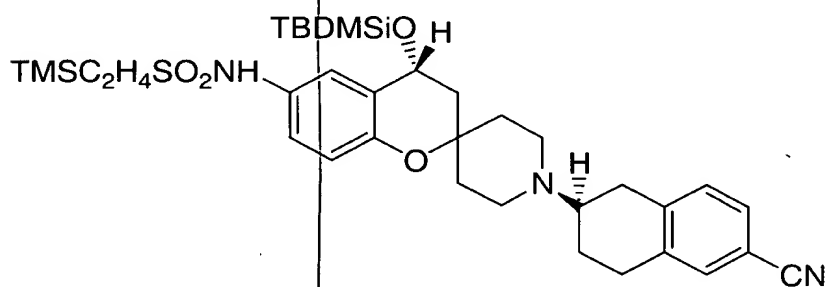


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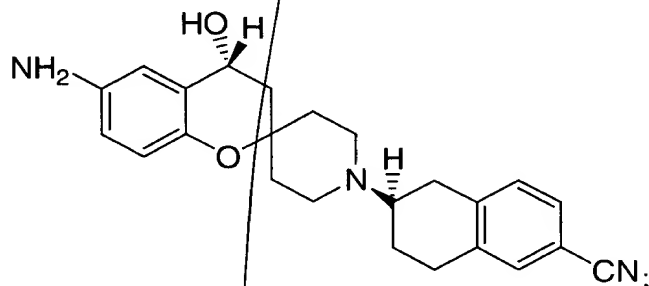
and then eluting off the polar isomer using a polar solvent system



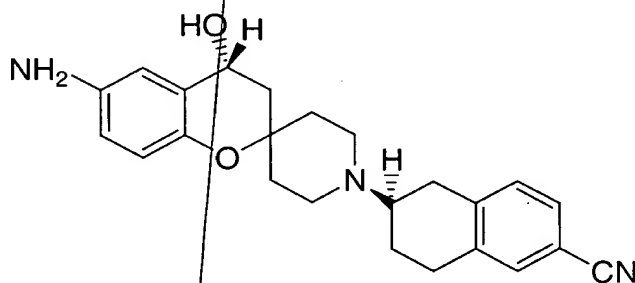
(5) desulfonating the non-polar isomer



using a fluoride compound in an organic base and heating for about 24 hours to about 48 hours to give the free alcohol-amine



- 5 (6) reacting the free alcohol-amine with trimethylsilyl imidazole in an organic solvent



to give the desired trimethylsilyloxy compound.

- 10 29. The process as recited in Claim 28, wherein the alkylation in step 2 is stirred at room temperature for up to 24 hours.

- 15 30. The process as recited in Claim 29, wherein the C₁-C₈-alkanethiolate in step 3 is sodium methanethiolate, sodium ethanethiolate, sodium propanethiolate or sodium 2-methylpropanethiolate.

31. The process as recited in Claim 30, wherein the desulfonation reaction in step 3 is run for less than 24 hours.

32. The process as recited in Claim 31, wherein the separation of the sulfonamide mixture in step 4 is run using flash chromatography with a solvent system of ethyl acetate and hexane to ethyl acetate and methanol.

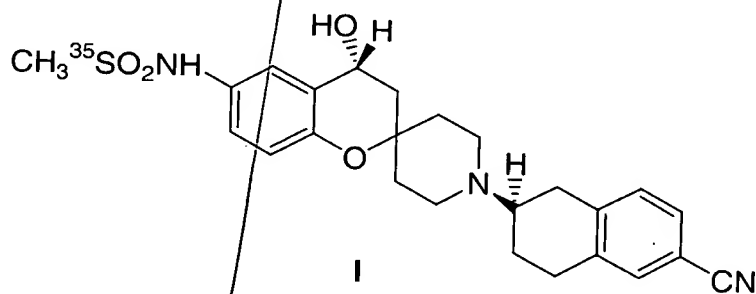
33. The process as recited in Claim 32, wherein the separation of the sulfonamide mixture in step 4 is run using flash chromatography with a non-polar solvent system of 1:1 ethyl acetate: hexane to a polar solvent system of 99:1 ethyl acetate: methanol.

34. The process as recited in Claim 33, wherein The fluoride compound used in the desulfonylation of step 5 is selected from: cesium fluoride and tetrabutylammonium fluoride.

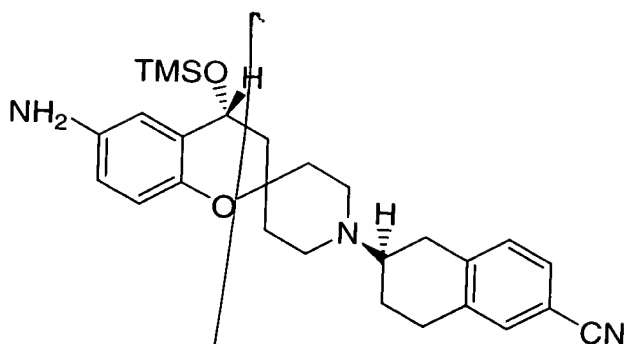
35. The process as recited in Claim 34, wherein the organic solvent used in the desulfonylation of step 5 is selected from: dimethylformamide, dimethylsulfoxide and N-methylpyrrolidinone.

36. The process as recited in Claim 35, wherein the organic solvent in step 6 is acetonitrile, tetrahydrofuran, or ether.

37. A process for the preparation of a radioligand compound of Formula I



comprising the steps of:
(a) reacting the amine



with [^{35}S]-methanesulfonyl chloride in the presence of an organic base to form the silyl-protected [^{35}S]-methanesulfonamide; and

(b) removing the silyl-protecting group of the silyl-protected [^{35}S]-methanesulfonamide with trifluoroacetic acid to give the radioligand compound of Formula I.

38. The process as recited in Claim 37, wherein the organic base is selected from triethylamine, trimethylamine, and diisopropylethylamine.

39. The process as recited in Claim 38, wherein the organic base is triethylamine.

40. A compound which is

